The influence of soluble carbon and fertilizer nitrogen on nitric oxide and nitrous oxide emissions from two contrasting agricultural soils

Laura Sánchez-Martín , Antonio Vallejo , Jan Dick , Ute M Skiba

Department of Agricultural Chemistry, Polytechnic University of Madrid, Ciudad Universitaria, s/n 28040 Madrid, Spain
Centre for Ecology and Hydrology, Edinburgh, Bush Estate, Penicuik, Midlothian EH26 QB, UK

Abstract

Contradictory effects of simultaneous available organic C and N sources on nitrous oxide (N_2O), carbon dioxide (CO_2) and nitric oxide (NO) fluxes are reported in the literature. In order to clarify this controversy, laboratory experiments were conduced on two different soils, a semiarid arable soil from Spain (soil I, pH = 7.5, 0.8%C) and a grassland soil from Scotland (soil II, pH = 5.5, 4.1%C). Soils were incubated at two different moisture contents, at a water filled pore space (WFPS) of 90% and 40%. Ammonium sulphate, added at rates equivalent to 200 and 50 kg N ha^{-1}, stimulated N_2O and NO emissions in both soils. Under wet conditions (90% WFPS), at high and low rates of N additions, cumulative N_2O emissions increased by 250.7 and 8.1 ng N_2O–N g^{-1} in comparison to the control, respectively, in soil I and by 472.2 and 2.1 ng N_2O–N g^{-1}, respectively, in soil II. NO emissions only significantly increased in soil I at the high N application rate with and without glucose addition and at both 40% and 90% WFPS. In both soils additions of glucose together with the high N application rate (200 kg N ha^{-1}) reduced cumulative N_2O and NO emissions by 94% and 55% in soil I, and by 46% and 66% in soil II, respectively. These differences can be explained by differences in soil properties, including pH, soil mineral N and total and dissolved organic carbon content. It is speculated that nitrifier denitrification was the main source of NO and N_2O in the C-poor Spanish soil, and coupled nitrification–denitrification in the C-rich Scottish soil.

Keywords: Nitrous oxide; Nitric oxide; Soil respiration; Mineral N; Glucose; Soil moisture; Mitigation

1. Introduction

Agricultural soils are significant sources of nitrous oxide (N_2O), nitric oxide (NO), and carbon dioxide (CO_2) (IPCC, 2001). The observed emissions of these gases are the result of two counteracting phenomena: the production and consumption by soil (Duxbury et al., 1993). N_2O and NO are produced predominantly by microbial processes, as by-products of nitrification and products of denitrification (Firestone and Davidson, 1989). The balance between these processes varies with climate, soil conditions and soil management (Skiba et al., 1997; Skiba and Smith, 2000). Nitrogen fertilizer application to agricultural soils generally increases N oxides emission (Chadwick et al., 2000). Fertilizer type has been shown to influence N_2O and NO emission rates (Mosier et al., 1998; Vallejo et al., 2006). Additions of NH_4-based fertilizers and urea to aerated soils provide substrates for nitrification, and the product of nitrification, nitrate (NO_3^-), will provide a substrate for denitrification. The associated increases in nitrification and denitrification rates and accompanying N_2O and NO losses tend to be short-lived, lasting from a few days to several weeks depending on the management of the agroecosystem (Skiba and Smith, 2000). Organic fertilizers not only supply mineral N, but also organic C. These C additions stimulate general heterotrophic activity in the soil, including denitrification. Increased respiration rates will increase O_2 consumption and further promote the anaerobic conditions required for denitrification to flourish (Cannavo et al., 2003). Increased N_2O emissions were measured from intensively managed grassland plots fertilized with manures compared to adjacent plots fertilized with mineral N (Ding et al., 2007). In contrast, after a heavy rainfall event, a

reduction in N_2O emissions from plots receiving organic fertilizers compared to mineral fertilizers were observed for a Scottish grassland (Ball et al., 2004).

In order to understand the influence of the addition of soluble organic C and mineral N on N_2O and NO emissions, laboratory experiments were carried out using two contrasting soils: a high carbon content grassland soil and in a low carbon content semiarid arable soil. The effect of N fertilizer rate, with and without additions of a simple carbon source (glucose) on NO, N_2O and CO_2 emissions was studied under dry and wet soil conditions.

2. Material and methods

2.1. Soils

The experiments were conducted on two soils with different organic matter content. Soil I with a clay loam texture was located at 'El Encín' Field Station, near Alcalá de Henares (Madrid, Spain) (latitude 40° 32′N, longitude 3° 17′W), in the middle of the Henares river basin. In April 2006, 5 kg of soil were collected randomly from a 700 m^2 area and was mixed. This soil had not been fertilized and cultivated for at least two years and was without vegetation when collected. The soil was dried in the laboratory at atmospheric temperature and transported by air to CEH, Edinburgh, Scotland. Soil II, also a clay loam, was located at Easter Bush, 10 km south of Edinburgh in Scotland (latitude 55°52′N, longitude 3°2′W). The field is a managed grassland (>90% *Lolium perenne*), grazed by sheep and cattle and is periodically cut for silage. Some physico-chemical properties of the 0–28 cm top soil layer of soil I and II are shown in Table 1.

2.2. Experimental details

Four successive laboratory experiments using the same treatments were carried out in following order: (1) soil I (arable soil with low organic C content) at a high WFPS (90%); (2) soil I at low WFPS (40%); (3) soil II (grassland soil with high organic C content) at high WFPS (90%); (4) soil II at low WFPS (40%).

For each experiment, aliquots of 100 g of each soil were placed into 15 clear Perspex columns (20 cm height × 5 cm diameter), which were sealed at the base and during flux measurements also at the top with a plastic lid and insulating tape. The headspace of the tubes was 0.341 l. Gas inlet and outlet holes (0.5 cm) were fitted with three way taps; the outlet hole was 5 cm above the inlet (Dick et al., 2001). Air tightness was tested by inserting a known concentration of N_2O and CO_2 and incubation for one hour. The gas concentration during the incubation period was maintained.

Soils were wetted 10 days before the start of the experiment using 30 ml of distilled water for each aliquot to avoid the interference of pulses of NO and N_2O typically observed as a consequence of the first wetting of dry soil (Dick et al., 2001). After this period, soil samples were rewetted to obtain the 40% or 90% WFPS required for the experiments. The WFPS was maintained throughout the experimental period, by daily reweighing of the cores and replacing weight losses with distilled water.

For analysis of dissolved organic C (DOC) and mineral N soil content aliquots of 15 g of soil were placed in identical containers, as those used for the flux cores, and were treated in the same way. These additional aliquots were used in order not to destroy the flux cores. All soils were incubated in the laboratory at a constant air temperature of 25.5 ± 0.9 °C, the optimal temperature for most soil microbial processes.

The applied treatments were: (i) high mineral N concentration, (0.40 mg N g^{-1} soil, which is equivalent to approximately 200 kg N ha^{-1} (HN); (ii) low mineral N concentration (0.1 mg N g^{-1}soil, which is equivalent to 50 kg N ha^{-1} (LN); (iii) glucose (1%) together with high mineral N concentration (Glu + HN) and (iv) glucose (1%) together with low mineral N concentration (Glu + LN) (v) control without any added N or C compounds (Control). Mineral N was added as $(NH_4)_2SO_4$. Both, mineral N and glucose were applied to soil dissolved in the distilled water, used to obtain the desired WFPS values. All treatments were replicated 3 times.

2.3. Trace gas flux measurements

Trace gas fluxes were measured daily for the first 5 days after treatment and then less frequently for the following 15–18 days. Nitric oxide fluxes were measured from the soil columns using a gas flow-through system, as described in detail by Dick et al. (2001). The columns were closed for 20 min. During this period, ambient air, filtered through charcoal and aluminium/$KMnO_4$, to remove O_3 and NO_x,

Table 1
Some physico-chemical properties of the 0–28 cm top soil layer for both soils measured by conventional methods

	Total organic C (g kg^{-1})	Total N (g kg^{-1})	pH_{H_2O}	Bulk density (mg m^{-3})	Clay (%)	Silt (%)	Sand (%)	Dissolved organic C (DOC) (mg C kg^{-1})	NO_3^- (mg NO_3^--N kg^{-1})
Soil I	8.2	0.7	7.5	1.41	28[a]	17	55	35	12.3
Soil II	35.4	2.6	5.5	1.40	21[b]	27	52	195.7	0.8

[a]Predominately as vermiculite.
[b]Predominately as kaolinite.

was passed over the headspace of the column at a flow rate of 40 ml min^{-1}. NO was analyzed by chemiluminescense using a 42C NO_x analyzer Thermo Environmental Instruments. A dual channel photometric ozone analyzer (Thermo Environmental Instruments, model 427) was used to measure O_3. Both analyzers require a flow rate of 1 l min^{-1} each, so additional filtered air was supplied to the analyzer and the dilution of the sample air was calculated from the flow rate recorded using a mass flow meter (Aera FC 7700C). NO and O_3 concentrations, air temperature and flow rates were recorded at 10 s intervals on a datalogger (Campbell Scientific 21*x*). Flux measurements from soil columns were interspersed with three measurements from an empty column, in order to take into account reactions with the chamber walls and lids. The NO flux (ng N g^{-1} h^{-1}) was calculated as the product of the flow rate of the air stream through the repacked soil column, the increase in NO concentration above the control (empty column) and the dilution rate divided by the dry weight of soil (100 g).

Nitrous oxide and carbon dioxide fluxes were measured in samples extracted from the headspace of the soil columns after sealing for ~30 min. Gas samples (20 ml) were withdrawn by syringe and were transferred to pre-evacuated 20 ml vials. The vials were prepared by repeated (5 times) evacuation to −80 bar and then flushed with N_2, to removed ambient air, and were stored at −80 kPa for maximum of 24 h before being filled with the sample air. The samples were analyzed within 1 day. All gases were analyzed by gas chromatography (Hewlett Packard 5890) using an autosampler (Varian Genesis). A flame ionization detector fitted with a methanizer was used to measure CO_2 and an electron-capture detector to measure N_2O.

Ambient room air was collected at the beginning of each sample set. Fluxes of N_2O and CO_2 were calculated as the product of the increase in concentration above ambient laboratory air and volume of the headspace in the repacked soil column divided by the time the column was sealed and the dry weight of the soil.

2.4. Soil analysis

Soil NO_3^- and NH_4^+ content from each column (at the end of the experiment) and from the additional container (the day following to start the experiment) were determined by extracting 15 g of fresh soil with 50 ml 1 M KCl solution; NO_3^- and NH_4^+ were analyzed by colorimetric methods (Harwood and Huysen, 1970; Henriksen and Selmer-Olsen, 1970). Soluble organic C was also extracted and analyzed in samples obtained at the end of the experiment as described by Mulvaney et al. (1997).

Gravimetric moisture contents for the columns were derived from the relationship between wet weight of the soil column and the dry weight of the soil column. Water filled pore space (WFPS) was calculated by dividing the volumetric water content by total soil porosity. Total soil porosity was calculated according to the relationship: soil porosity = (1−soil bulk density/2.65), assuming a particle density of 2.65 g cm^{-3}. Bulk densities were calculated from the volume of soil in the cores.

2.5. Statistical analysis

The statistical analysis was performed using STATGRAPHICS Plus 5.1 (Manugistics, 2000). Least significant difference tests (LSD) were used for comparison of means between treatments. A multifactor ANOVA was used to establish the influence of added N, added C, WFPS and type of soil on N_2O, CO_2 and NO emissions, DOC, NH_4^+-N and NO_3^--N content in soil.

3. Results

3.1. N_2O emission

The application of $(NH_4)_2SO_4$ produced different N_2O fluxes, depending on the rate of fertilizer, type of soil, moisture content and the addition of glucose (Fig. 1). For the semiarid arable soil (soil I), largest fluxes of N_2O were observed from samples treated with N fertilizer equivalent to a rate of 200 kg N ha^{-1}. In the period 2–12 days after the start of the experiment, N_2O fluxes ranged from 13.4 to 49.9 ng N_2O-N g^{-1} h^{-1} at 90% WFPS and from 0.6 to 4.3 ng N_2O-N g^{-1} h^{-1} at WFPS of 40% (Fig 1). For the entire experimental period, the total cumulative N_2O emission for this fertilizer treatment was 251.7 and 21.7 ng N_2O-N g^{-1} for the high and the low WFPS rates, respectively (Table 2). The addition of glucose significantly reduced N_2O emissions ($P<0.05$) in cores that received a high mineral N by 94% at the high WFPS and by 22% at the low WFPS. At the low N rate additions of glucose did not significantly reduce N_2O emissions.

In the high organic C content grassland soil (soil II) emissions of N_2O were generally much larger than from soil I (Fig. 1). Cumulative emissions ranged from 598.6 to 1438.4 ng N_2O-N g^{-1} at 90% WFPS and between 3.7 and 84.4 ng N_2O-N g^{-1} at 40% WFPS (Table 2). Even cumulative emissions from the soil II control columns (966.2 ng N_2O-N g^{-1}) were several orders of magnitude larger than those from soil I (0.9 ng N_2O-N g^{-1}) (Table 2). With additions of glucose N_2O emissions peaked on the second day after the start of the experiment, both at 90% WFPS (358.3 and 456.7 ng N_2O-N g^{-1} h^{-1} for Glu + HN and Glu + LN, respectively) and 40% WFPS (69.2 and 84.3 ng N_2O-N g^{-1} h^{-1} for Glu + HN and Glu + LN, respectively). After this day N_2O emission decreased rapidly, and at 90% WFPS were significantly smaller than from control columns between days 4–11 ($P<0.05$). Analysis of variance in both soils showed that N_2O emissions were positively influenced by high WFPS, although the effect was more significant in soil II ($P< 0.001$) than in soil I ($P<0.05$). Glucose additions reduced N_2O emissions significantly in soil I ($P<0.01$), whereas N additions significantly stimulated N_2O emissions in both soils ($P<0.05$).

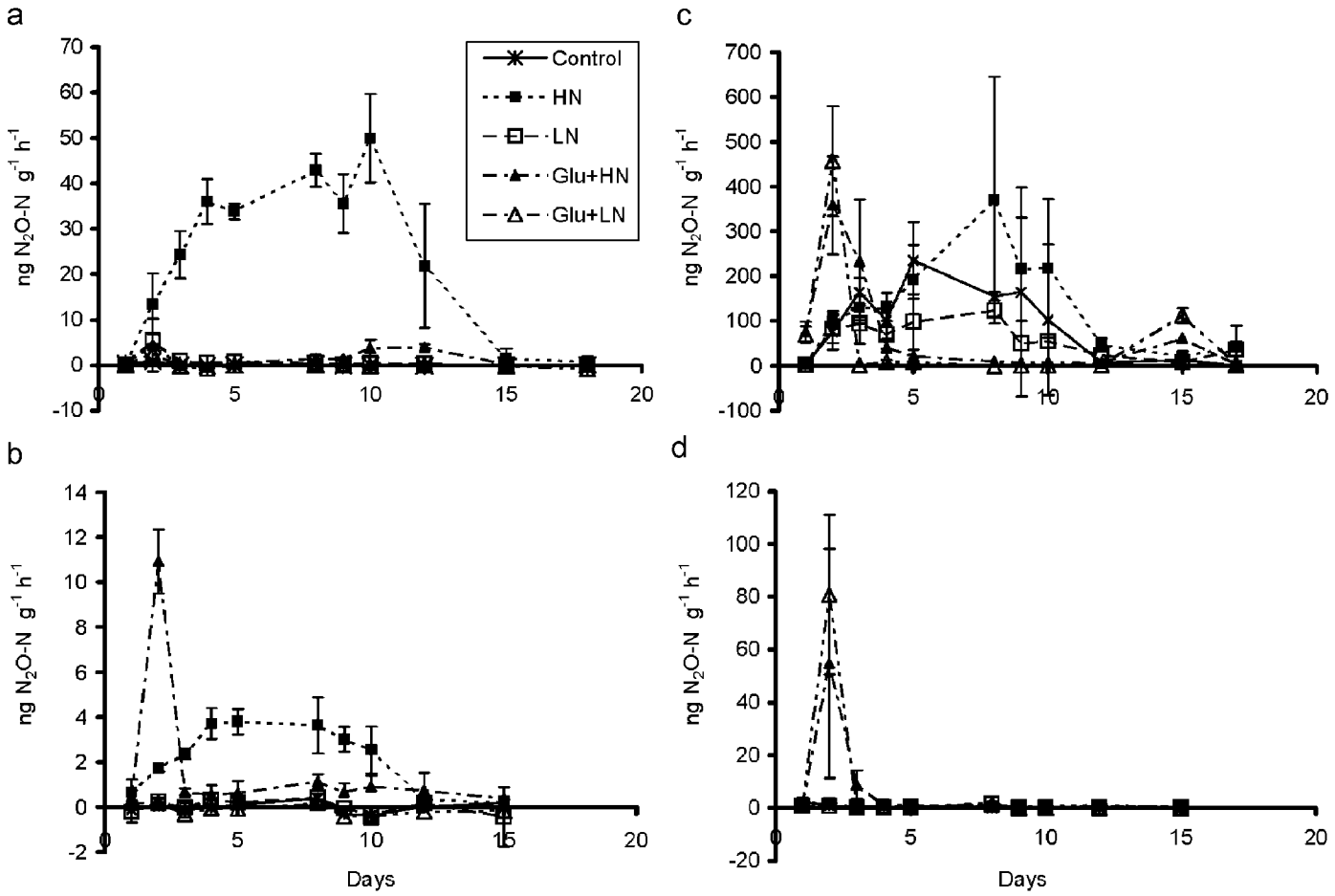

Fig. 1. Emissions of N_2O from soil columns, after addition of glucose (glu) with and without $(NH_4)_2SO_4$ at rates equivalent to 50 (LN) and 200 (HN) kg N ha^{-1} and a control (only water applied). (a) soil I at 90% WFPS; (b) soil I at 40% WFPS; (c) soil II at 90% WFPS and (d) soil II at 40% WFPS. The vertical bars indicate standard error. The *y*-axis of graphs (a)–(d) are at different scales.

Table 2
Cumulative N_2O, NO and CO_2 fluxes emitted for soil I (low organic carbon content, Spain) and soil II (high organic carbon content, Scotland) treated with high and low mineral N with and without glucose (Glu) additions and incubated at 90% and 40% WFPS

Treatment	N_2O (ng N_2O-N g^{-1})		NO (ng NO-N g^{-1})		CO_2 (μg CO_2-C g^{-1})	
	Soil I	Soil II	Soil I	Soil II	Soil I	Soil II
90% WFPS						
Control	0.92 a	966.21 ab	−0.13 a	0.19 a	9.40 a	24.72 a
HN	251.67 b	1438.38 b	73.87 c	3.52 b	30.64 a	31.10 a
LN	9.02 a	625.72 a	2.01 a	2.79 ab	6.30 a	26.34 a
Glu + HN	13.72 a	777.46 ab	33.09 b	1.19 ab	222.36 b	266.15 b
Glu + LN	−0.43 a	598.66 a	−0.04 a	0.52 ab	190.71 b	280.23 c
40% WFPS						
Control	−0.22 a	5.04 a	0.27 a	4.29 ab	−15.48 a	3.10 a
HN	21.65 c	9.70 a	26.85 c	11.02 b	−2.23 b	4.32 a
LN	0.24 a	3.76 a	0.98 a	6.19 ab	−17.71 a	−18.76 a
Glu + HN	16.77 b	69.19 b	7.68 b	5.99 ab	162.83 c	191.63 b
Glu + LN	−1.26 a	84.36 b	1.49 a	0.51 a	164.62 c	236.67 c

Data shown are means of three replicate samples. Different letters within columns indicate significant differences at $P<0.05$ using LSD test.

3.2. NO fluxes

In soil I, the largest rates of NO emissions were measured from the high rate of mineral N treatment at 90% WFPS (37.0 ng NO-N g^{-1} h^{-1}) on the 10th day (Fig. 2) and at 40% WFPS (7.6 ng NO-N g^{-1} h^{-1}) on the 5th day after the start of the experiment (Fig. 2). Reducing the rate of N fertilizer strongly reduced NO fluxes under

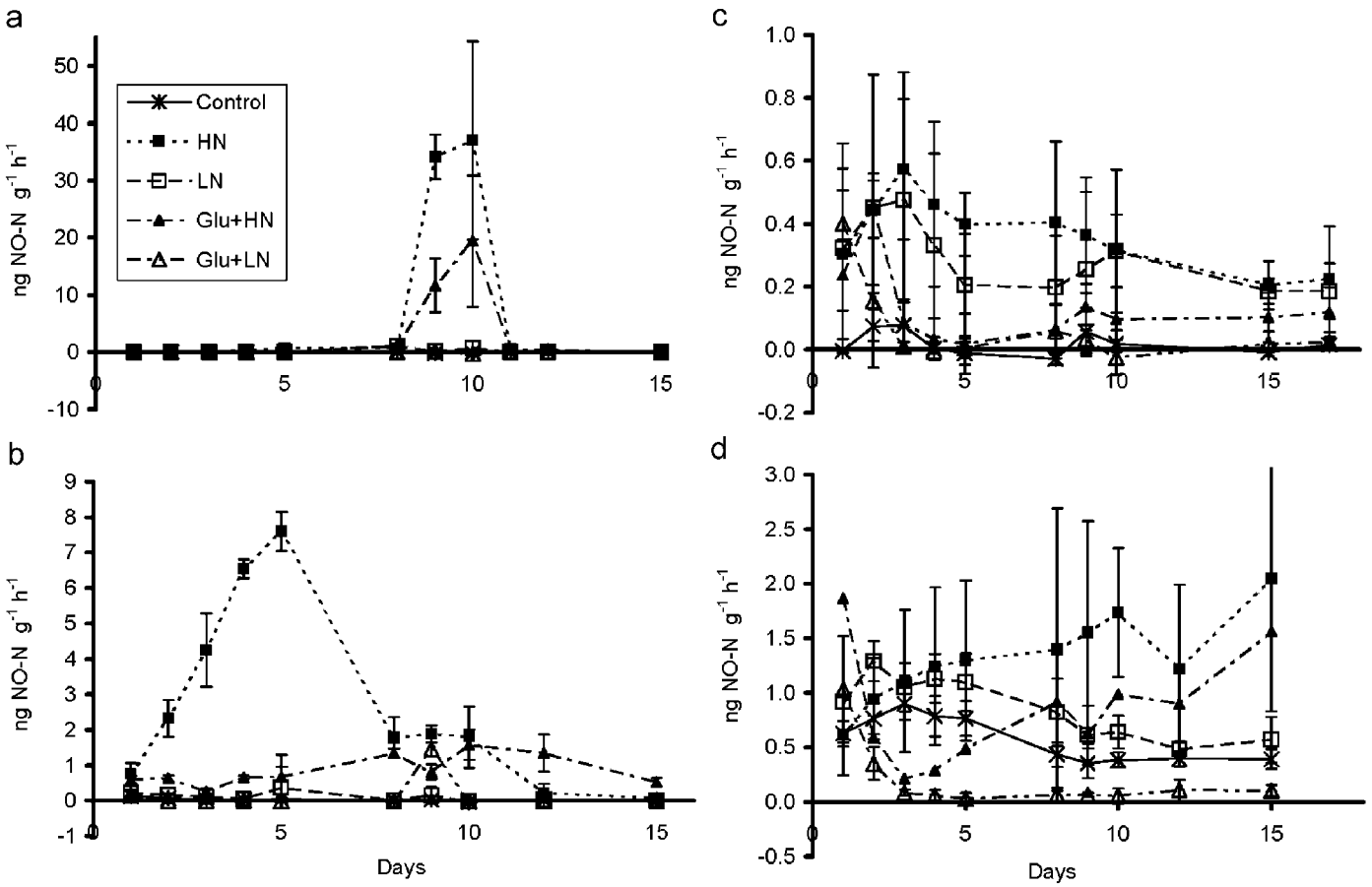

Fig. 2. Fluxes of NO from the soil during the experimental period. (a) Soil I at high WFPS; (b) soil I at low WFPS; (c) soil II at high WFPS and (d) soil II at low WFPS. The vertical bars indicate standard error. The *y*-axis of graphs (a)–(d) are at different scales.

both soil moisture conditions. For the high mineral N treatment additions of glucose reduced cumulative NO emissions by 55% at 90% WFPS and by 71% at 40% WFPS (Fig. 2). As for N_2O emissions, the addition of glucose together with the low N application rate had a limited effect on reducing NO fluxes in this soil.

In contrast to the N_2O fluxes the grassland soil II emitted smaller NO fluxes than the semiarid arable soil I (Fig. 2). NO emissions for the soil II ranged from -0.025 to $2.04\,ng\,NO\text{-}N\,g^{-1}\,h^{-1}$ across the two moisture conditions (Fig. 2), although in general NO emission was larger at the smaller WFPS (40%) (Table 2). The addition of glucose reduced total NO emission at high and low N application rates, but these reductions were not significant ($P > 0.05$)

Analysis of variance in both soils showed that NO emissions were stimulated to a greater extent by mineral N additions in soil I ($P<0.001$) than in soil II ($P<0.01$). Glucose additions reduced NO emissions in both soils ($P<0.05$), whereas WFPS stimulated NO emissions in soil I ($P<0.01$) but reduced emissions in soil II ($P<0.001$).

3.3. CO_2 emissions

Soil respiration increased significantly ($P<0.05$) when soils I and II were treated with glucose (Fig. 3). At 90% WFPS, largest fluxes of CO_2 (from 52 to $56\,\mu g\,CO_2\,g^{-1}\,h^{-1}$) occurred between 2 and 5 days after glucose addition, for soils I and II, respectively (Fig. 3). At 40% WFPS, CO_2 emissions peaked on the 2nd day after the start of the experiment, measuring fluxes of 92.6 and $55.4\,\mu g\,CO_2\,g^{-1}\,h^{-1}$ for Glu + HN and Glu + LN, respectively, in soil I, and 75.2 and $101.2\,\mu g\,CO_2\,g^{-1}\,h^{-1}$ for the same treatments in soil II. Applications of mineral N at the low and high rate did not significantly influence soil respiration rates ($P>0.05$), although larger emissions were detected at 90% WFPS than at 40% WFPS. In general, average soil respiration rates were higher in soil II than in soil I (Table 2). The differences were larger at 40% WPFS, where soil I showed negative total CO_2 fluxes when glucose was not added, probably as consequence of CO_2 consumption by soil microflora.

3.4. DOC and mineral N concentrations

The application of $(NH_4)_2SO_4$ without glucose decreased the DOC concentration, measured at the end of the experimental period, compared to the control (Table 3). This reduction of DOC was higher for the high N rate than the low N rate, and significant differences at $P < 0.05$ were observed between high N and control in all cases, except in

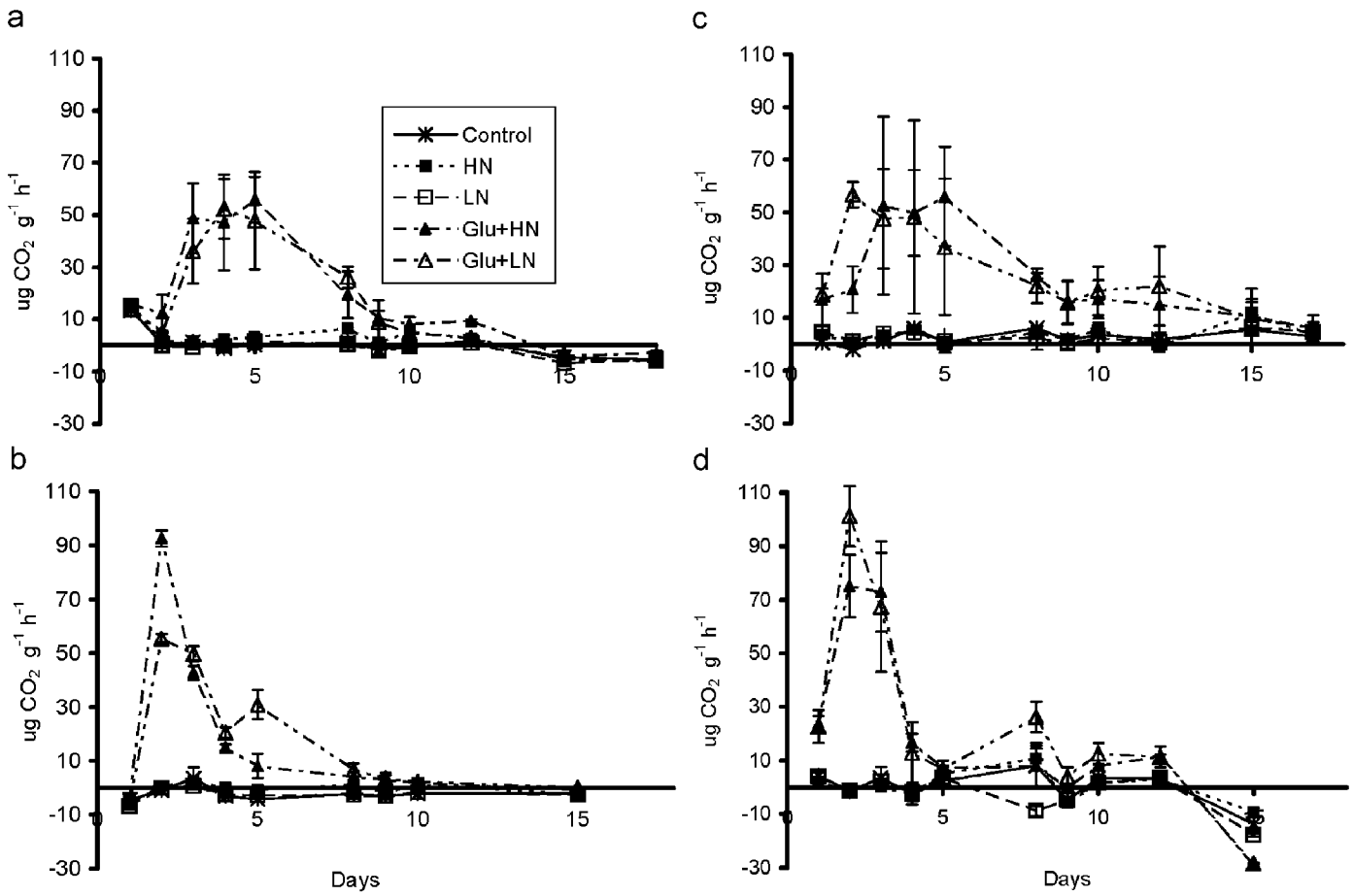

Fig. 3. Fluxes of CO_2 from soil during the experimental period. (a) Soil I at high WFPS; (b) soil I at low WFPS; (c) soil II at high WFPS and (d) soil II at low WFPS. The vertical bars indicate standard error.

Table 3
Dissolved organic carbon contents at the end of the experiments at high WFPS (90%) and low WFPS (40%) from soils with low (soil I) and high (soil II) organic carbon content treated with high and low mineral N with and without glucose (Glu) addition

Treatment	90% WFPS DOC ($mg\,C\,kg^{-1}$)		40% WFPS DOC ($mg\,C\,kg^{-1}$)	
	Soil I	Soil II	Soil I	Soil II
Control	61 b	192 d	59 a	76 bc
HN	38 a	74 ab	40 a	61 a
LN	41 ab	82 bc	45 a	64 ab
Glu + HN	29 a	52 a	40 a	83 c
Glu + LN	61 b	109 c	89 b	100 d

Data shown are means of three replicate samples. Different letters within columns indicate significant differences at $P<0.05$ using LSD test.

soil I at 40% WFPS. In both soils reductions were largest for the glucose and high N treatment compared to the high N treatment at 90% WFPS. For the glucose and low N treatment a significant reduction in DOC was produced in soil II at 90% WFPS.

The NH_4^+ added as fertilizer was reduced during the experimental period (15 days) in soil I, and final values, smaller than $3.1\,mg\,NH_4^+$-$N\,kg^{-1}$, were recorded in all treatments at both WFPS 90% and 40% (Table 4). In contrast, the decrease in NH_4^+ was much smaller in soil II at 90% WFPS. Under aerobic conditions (WFPS = 40%) soil II maintained the highest concentrations of NH_4^+ in the high N ($256\,mg\,NH_4^+$-$N\,kg^{-1}$) and glucose and high N treatments ($175\,mg\,NH_4^+$-$N\,kg^{-1}$) at the end of the experiment.

In soil I, glucose and high N and high N without glucose treatments increased the soil NO_3^- content at the end of the experimental period, whereas the control, glucose and low N, and low N without glucose maintained or reduced NO_3^- content (Table 4). In soil II, soil NO_3^- content increased for the mineral treatments ($\Delta NO_3^- = 15\,mg\,NO_3^-$-$N\,kg^{-1}$ for high N and $18\,mg\,NO_3^-$-$N\,kg^{-1}$ for low N application) at WFPS of 90%, and for all treatment for drier conditions (WFPS = 40%). At 40% WFPS only $\Delta NO_3^- = 7\,mg\,NO_3^-$-$N\,kg^{-1}$ was produced when mineral N was added with glucose (Glu + LN).

Table 4
NO_3^- and NH_4^+ concentrations in soil I and soil II one day after addition of fertilizers (values in parenthesis) and at the end of the experimental period (values without parenthesis)

Treatment	90% WFPS				40% WFPS			
	NO_3^- (mg NO_3^--N kg^{-1})		NH_4^+ (mg NH_4^+-N kg^{-1})		NO_3^- (mg NO_3^--N kg^{-1})		NH_4^+ (mg NH_4^+-N kg^{-1})	
	Soil I	Soil II	Soil I	Soil II	Soil I	Soil II	Soil I	Soil II
Control	2.1 a	0.2 a	1.7 bc	46.7 b	12.4 ab	39.3 bc	0.5 ab	5.4 a
	(5.9)	(6.9)	(3.2)	(55.6)	(13.4)	(5.5)	(24.7)	(64.8)
HN	71.2 c	21.6 a	0.6 a	256.0 d	102.9 d	42.3 c	0.0 a	256.2 c
	(10.6)	(6.1)	(277.7)	(318.8)	(13.4)	(5.4)	(27.3)	(361.1)
LN	7.8 a	25.1 a	1.1 ab	27.7 ab	14.9 b	43.6 c	0.0 a	11.8 a
	(8.8)	(6.4)	(16.3)	(68.5)	(13.4)	(5.0)	(27.5)	(67.8)
Glu + HN	54.0 b	2.3 a	1.6 bc	170.7 c	42.3 c	33.1 b	3.1 b	175.1 b
	(9.7)	(6.3)	(147.0)	(193.6)	(13.4)	(5.2)	(27.4)	(214.4)
Glu + LN	1.9 a	0.1 a	2.1 c	13.5 a	0.0 a	12.3 a	0.0 a	1.2 a
	(9.2)	(6.3)	(81.6)	(131.0)	(13.4)	(5.1)	(27.4)	(141.1)

Data shown are means of three replicate samples. Different letters within columns indicate significant differences at $P < 0.05$ using LSD test. The standard error was usually <5% of the mean in all cases.

4. Discussion

4.1. Soil properties

In this study we have compared NO, N_2O and CO_2 fluxes from typical Scottish grazed grassland and a typical Spanish semiarid arable soil. The same laboratory treatments stimulated very different patterns of NO and N_2O fluxes, due to differences in chemical and physical soil properties, especially total and soluble soil carbon, total nitrogen content and soil pH (Table 1). The Scottish cool temperate climate promotes carbon accumulation, whereas fast microbial turnover in the warmer semiarid Spanish climate does not conserve carbon at the same rate (Conant and Paustian, 2002). In addition carbon content and acidity of grassland soils is generally larger than that of an arable soil (Chantigny, 2003). DOC concentrations were also smaller in the Spanish than Scottish soil, and the addition of an N fertilizer further reduced the DOC concentration in soil (Table 3). Chantigny et al. (1999) also reported that the DOC content decreased markedly soon after applying 180 kg N ha^{-1} as NH_4NO_3. They suggested that N fertilization enhances DOC consumption by soil microbes, thereby decreasing its concentration. However, in our soils, the addition of inorganic N did not stimulate a significant change in soil respiration (Fig. 3), suggesting that DOC was not necessarily mineralized. Possibly, a fraction of DOC was immobilized into microbial biomass or released to the soil as microbial metabolites.

The larger organic N pool of the Scottish soil promoted higher mineralization rates and resulted in NH_4^+ concentrations 10 times larger than in the Spanish control soil columns 1 day after the start of the experiment. After addition of fertilizers, NH_4^+ disappeared more rapidly in soil I than in soil II. This difference could partly be explained by differences in the clay mineralogy. Soil I was dominated by vermiculite, that favored the rapid NH_4^+ fixation and explained the little NH_4^+ recovered the day after fertilization in this soil. Contrary, the C-rich soil II was dominated by kaolinite, where fixation was very limited (Scherer, 1993). In addition the faster loss of NH_4^+ from soil I could be also justified by larger nitrification rates. At the end of the experiment NO_3^- concentrations in the HN treatment were larger in the Spanish soil than in the Scottish soil (Table 4). Even at 90% WFPS nitrification seems to be occurring in both soils. In particular in the Spanish soil the small amount of O_2 at 90% WFPS did not appear to have reduced the rate of nitrification. These results confirm that nitrification can occur in wet soils (Adams and Akhtar, 1994). The differences in soil pH between soil I (pH 7.5) and soil II (pH 5.5) may also be a reason for the different autotrophic nitrification rates. Autotrophic nitrification is often considered to be sensitive to acidity, and reports consistently refer to optimum values in the range pH 6.5–8.0. Autotrophic nitrifiers can adapt to acidic environments, but possibly operate not as efficiently as under more alkaline conditions (Yamulki et al., 1997).

4.2. Emissions after addition of inorganic N

The observed emissions of N_2O and NO were the result of the production, consumption and transport of N_2O and NO in and out of the soil. The production and consumption of these gases mainly results from microbial transformations and depends on soil properties, climatic conditions and composition of N fertilizers added to soil. N_2O emissions were much larger from soil II than soil I at high WFPS (90%) (Table 2). The larger emission, even observed in the control of soil II (966.2 ng N_2O-N g^{-1}) in comparison to soil I (0.9 ng N_2O-N g^{-1}), could be explained by the fact that soil II had a larger total and soluble organic C content compared to soil I (Tables 1 and 3), which would favor denitrification and enhance soil respiration rates. The

control of soil II produced 2.6-fold more CO_2 than the control of soil I. In addition, in acidic soils the reduction of N_2O to N_2 was impaired, leading to an enhanced proportion of N_2O in the denitrification products (Thomsen et al., 1994).

Under dry conditions (WFPS = 40%), larger nitrification rates than under wet conditions (WFPS = 90%), were observed. The drier soil promotes nitrification, and it is likely that most of the N_2O and NO observed at 40% WFPS, especially in soil I, were produced by nitrifier denitrification. Nitrifiers switch from the oxidation of NH_4^+ to NO_3^- to the reduction of hydroxylamine and NO_2^- to NO and N_2O, when the O_2 supply diminishes. This process is stimulated by high concentrations of NH_4^+ and does not require an organic C source (Wrage et al., 2001).

Under wet conditions (90% WFPS), the addition of NH_4^+ without glucose had a different effect on N trace gas emissions in soil I than in soil II, and depended on the N application rate. The variation of cumulative N_2O flux compared to the control was +8 and −341 ng N_2O-N g^{-1} for the low N rate (equivalent to 50 kg N ha^{-1}) in soils I and II, respectively, but +250 and +472 ng N_2O-N g^{-1} for the high N rate (equivalent to 200 kg N ha^{-1}) in both soils, respectively. N_2O was probably a product of simultaneous nitrifier denitrification and denitrification. In soil I, nitrifier denitrification was likely to be an important source of NO and N_2O.

In both soils, the wetter conditions at 90% WFPS would have promoted the development of anaerobic microsites, suitable for denitrification. The composition of gases produced during denitrification depends on the oxidative state of the soil and the availability of organic C. Denitrifiers have a very high affinity for NO_3^- before utilizing N_2O, and similarly tend to utilize NO in preference to N_2O (Yamulki and Jarvis, 2002). Scholefield et al. (1997) demonstrated that the addition of NO_3^- to soil increased the N_2O/N_2 ratio in the emitted gases and NO_3^- also seems to inhibit the N_2O reductase enzyme (Blackmer and Bremner, 1978). The large differences in N_2O emissions between low N and high N application rates in soil I can be explained by large nitrification rates and accumulation of NO_3^- from HN treatments (71.2 and 102.9 mg NO_3^--N kg^{-1} at 90% and 40% WFPS, respectively) (Table 4). High NO_3^- concentrations partially inhibit the N_2O reductase enzyme during denitrification, therefore also the N_2O/N_2 ratio. The high NO_3^- soil content in the high N treatment of soil I, also explained the peaks of NO emissions observed 10 days following the fertilizer application. In that case denitrifiers have also utilized NO_3^- in preference of NO.

4.3. Emissions after addition of soluble organic C and mineral N

Glucose additions enhanced soil respiration rates in both soils. Respiration rates were not affected by the two N application rates, or by differences in pH of the Spanish and Scottish soils. The latter results were not in agreement with those of Sitaula et al. (1995), who observed decreased respiration rates in acid soils.

An interesting finding of this study is focused on the fact that NO and N_2O emissions from soils treated with glucose were lower than those from non-amended plots. This phenomenon seemed to be clearer at the highest N application rate. Significant reductions were observed in soil I. At 90% WFPS cumulative N_2O and NO emissions decreased by 95% and 55%, and at 40% WFPS by 23% and 71%, respectively. In soil II similar, but not statistically significant, reductions were observed (Table 2). Tiedje et al. (1983) suggested that organic C is more important than O_2 in stimulating the growth of denitrifier populations. The availability of C does not only support the activity of denitrifiers per se, but also has the indirect effect of causing microsite anaerobiosis, due to increased respiratory demand for O_2. Increased availability of labile C will favor complete denitrification to N_2. As nitrifier denitrification could be the dominant source of N_2O in the C-poor Spanish soil, applying a highly available C source, such as glucose, might have significantly depressed this process in favor of denitrification.

The only exception to the above was observed in soil II at 40% WFPS; glucose additions with low and high rates of N addition significantly increased N_2O emissions, rather than reducing these. It appears that for the acid (pH 5.5) high C Scottish soil glucose addition under aerobic conditions (40% WFPS) was not sufficient to stimulate complete denitrification to N_2. Similarly, increased denitrification rates and N_2O emissions where observed when a silty clay soil from Germany was treated with glucose and mineral N (Azam et al., 2002).

Our laboratory studies confirm the contrasting results observed in field studies comparing N trace gas fluxes after additions of mineral and organic soils. On a Scottish grassland, less than 5 km away from the field soil was collected for this study, Jones et al. (2005, 2007) compared N_2O emissions from plots treated with $(NH_4)_2SO_4$ and a range of organic fertilizers (poultry manure, cattle slurry), at rates of very high total N, but same rate of available N as the mineral treatment. The addition of manure and slurry stimulated denitrification and increased N_2O emissions. This observation is in agreement with the increased N_2O fluxes observed from the soil II cores treated with glucose and N at 40% WFPS. However, in an earlier similar field study on the same field, organic N applications at lower total N rates mitigated N_2O (Ball et al., 2004). Equally, the results presented here confirm the observations from plot experiments conducted on the same field from which soil was collected for this laboratory study (Vallejo et al., 2006), and that from different irrigated fields, also under Mediterranean conditions (Meijide et al., 2007). These authors found that the organic fertilizers (untreated pig slurry, digested thin fraction of pig slurry, composted pig slurry fraction and municipal solid waste) mitigated NO and N_2O emissions in irrigated semiarid soils

in comparison to the urea at the same available N rate and concluded that the reduction of N_2O/N_2 was dependent on the DOC content of the organic fertilizers. However, the laboratory study described here has demonstrated that a labile carbon source only reduced NO and N_2O emissions when applied together with mineral N. Research is required to select the optimal DOC content in organic N fertilizers to optimize the reduction of N_2O and NO from both C rich and C poor soils.

5. Conclusions

This study demonstrated that the addition of a labile carbon source together with mineral N fertilizers reduced the emissions of NO and N_2O in two contrasting soils: one low in organic carbon and alkaline pH and the second high in organic carbon content, but acid pH. This effect was especially effective in the low carbon semiarid soil, even at a low WFPS. Therefore, the use of organic instead of inorganic fertilizer could contribute to reduce emissions of NO and N_2O.

Different sources for NO and N_2O emissions between a C-rich and a C-poor soil were noticed. Emissions from the C-poor soil indicated that nitrifier denitrification was an important source of N oxides, while in the C-rich soil these compounds were produced by coupled nitrification–idenitrification.

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